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THE MICROSCALE WAR IN OUR WASTEWATER

Wastewater does not just contain organic pollutants – it often includes traces of pharmaceuticals, such as antibiotics, which can contribute to the development of bacterial antibiotic resistance.

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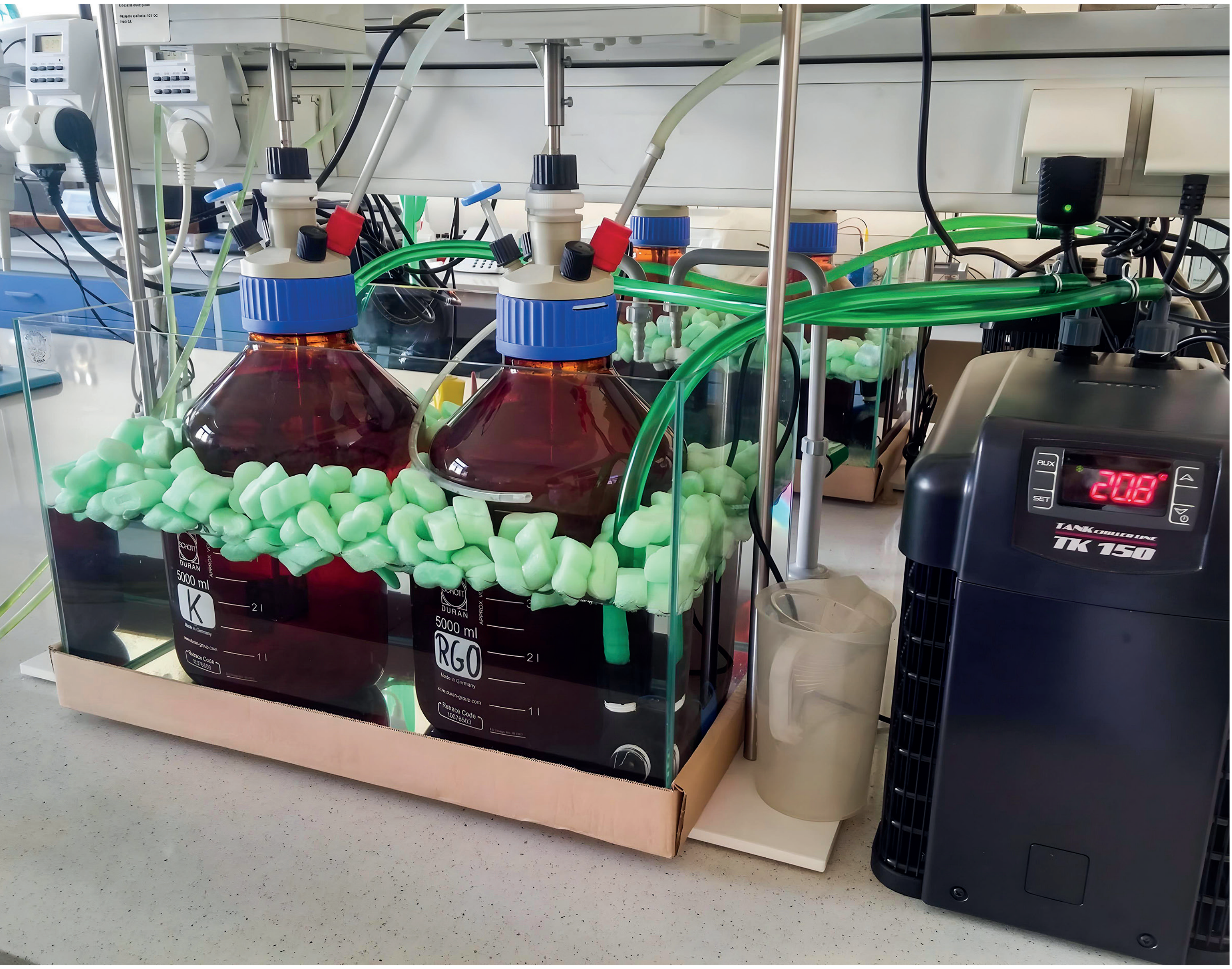
When people hear the word "biotechnology," they often think of medical applications, DNA manipulation, and genetic engineering. However, biotechnology extends far beyond medical advancements. One vital branch of the field, *environmental biotechnology*, focuses on improving the quality, protection, and health of our environment.

At its core, biotechnology relies on nature's intricate systems. By studying and adapting the strategies organisms use to survive and thrive, scientists create innovative technologies that enhance daily life. Yet, nature operates in cycles where everything is interconnected. This interconnectedness means that biotechnological solutions, while designed to address specific problems, often produce unintended consequences. These side effects remind us of the delicate balance required when applying science to natural systems.

One of the most critical biotechnological processes used on an industrial scale is biological wastewater treatment. This method is based on observations of the natural process of water self-purification, in which microorganisms remove pollutants from the environment. The efficiency of this natural process has been enhanced technologically through the use of concentrated microbial mixtures, known as *activated sludge*. Activated sludge contains bacteria, archaea, fungi, protozoa, and metazoans in the form of a dense, flocculated suspension. Microorganisms employed in wastewater treatment processes face threats, such as compounds that are byproducts of other biological transformations within the broader natural cycle of matter. These threats may also include substances beneficial to humans that, once released into the environment, pose risks to ecosystems and indirectly affect human health and well-being.

Biotechnological challenges

Removing pollutants from wastewater involves getting the elements that make up problematic chemical compounds involved in *biogeochemical cycles*. These cycles include the reactions and processes that facilitate the exchange of elements between the environment and living organisms. In wastewater treatment, particular focus is placed on the nitrogen and



A sequencing batch reactor system used to study the effects of antibiotics on nitrogen removal bacteria

phosphorus cycles, two biogenic elements essential for the structure and functioning of all life on Earth. Because nitrogen and phosphorus are abundant in the human body, their concentrations are particularly high in wastewater – mainly composed of substances derived from cellular metabolic processes. This makes removing these compounds before wastewater gets released into natural water bodies a top priority for treatment facilities. Efficient removal of nitrogen and phosphorus is crucial because these elements, while essential for growth, can cause over-fertilization of water bodies, known as eutrophication, when present in excess. Eutrophication often leads to algal blooms, especially in warmer months.

At first glance, the solution seems straightforward. Activated sludge contains large amounts of bacteria capable of processing significant quantities of nitrogen and phosphorus. Thanks to this, treated wastewater meets increasingly strict legal standards and poses no threat to the environment when discharged into rivers or lakes. However, our bodies metabolize more than just nutritious food. Many of us consume highly processed foods, stimulants, medications, and dietary supplements. Every ibuprofen tablet, for instance, passes through our bodies and eventually enters wastewater. As a result, wastewater treatment plants now face increasing quantities of hazardous substances known as micropollutants. These compounds, though present in very low concentrations, can have harmful effects on organisms. Common examples include anti-inflammatory drugs, hormones, and – particularly concerning for biological wastewater treatment – antibiotics and chemotherapeutics.

Antibiotics, both natural and synthetic, are a group of compounds widely used to treat bacterial infections. They are characterized by what is known as *selective toxicity*, meaning they kill bacteria while generally causing little to no harm to the cells of humans or animals undergoing treatment. The widespread adoption of antibiotics for treating bacterial diseases was considered a groundbreaking moment in medicine – a point when humanity seemed to have gained the upper hand over pathogenic bacteria and the ability to cure virtually any bacterial infection.

However, using antibiotics, a solution borrowed from nature, comes with certain challenging consequences. Bacteria under attack fight back by developing resistance to antibiotics. A resistant bacterium is unaffected by the antibiotic substance because it possesses mechanisms that effectively shield it from the harmful effects. It reproduces successfully, passing these resistance genes to its offspring. What's more, bacteria do not just share genetic material through parent-offspring inheritance, as humans do. Bacteria living within the same environment can exchange genetic information among themselves through a process called *horizontal gene transfer* (HGT), which plays a major role in the spread of antibiotic resistance in the environment.

> Rational antibiotic use and bacterial resistance are closely linked to biological wastewater treatment.

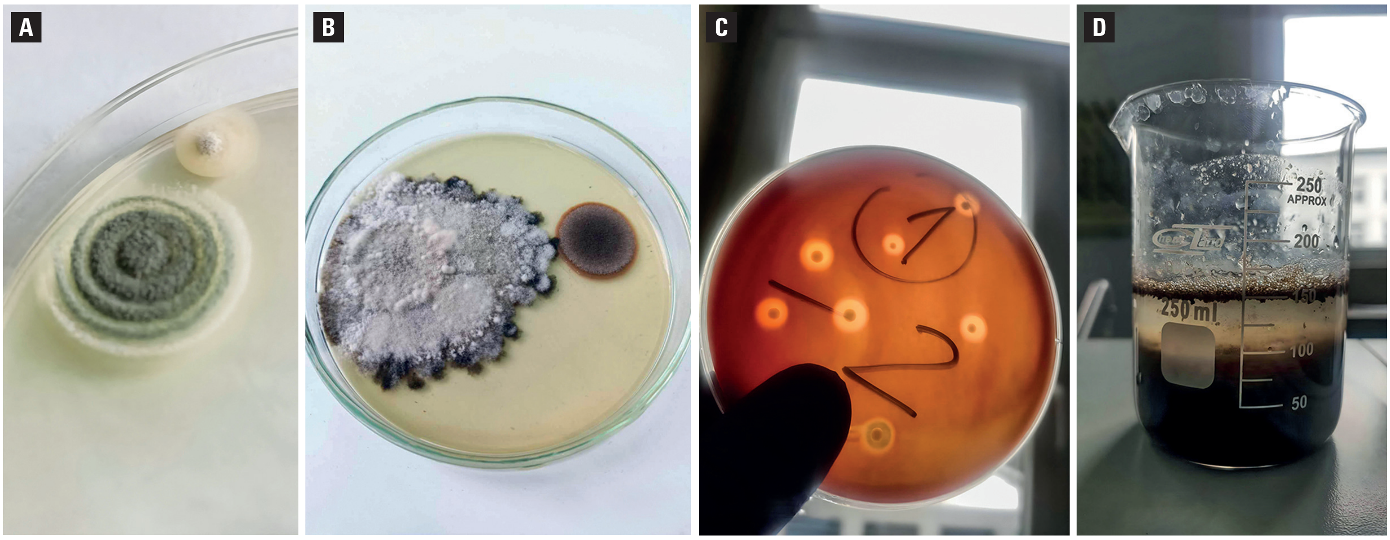
HGT can occur in a number of ways. The first is *conjugation*, where genetic material is transferred directly from one bacterial cell to another through physical contact. In this process, one cell donates genetic information, while the other receives it, gaining resistance to a specific antibiotic. Another method is *transformation*, in which a bacterium takes up genetic material directly from its surroundings. This allows living bacterial cells to acquire valuable genetic information from dead bacteria whose DNA remains in the environment. The third method is *transduction*, which involves bacteriophages – viruses that infect bacteria. Viruses are not living organisms and can replicate only by using a host cell. For a bacteriophage, the host is a bacterial cell. When a bacterium is infected, it produces new bacteriophages. Occasionally, these offspring viruses can "accidentally" carry fragments of the bacterial cell's DNA. If this DNA contains antibiotic resistance genes, the next bacterium infected by the virus will acquire this crucial information for its survival. One more form of HGT was recently discovered and is still not well understood. It involves the formation of outer membrane vesicles by Gram-negative bacteria (one of the two main groups of bacteria, which are distinguished by a cell wall covered by an additional outer membrane).

Problems with antibiotics

Every time bacteria are attacked with antibiotics, they respond. It's essentially an ongoing war, and we are all participants in this microbiological arms race. Whether we win or lose often depends on how rationally we use antibiotics, as our choices tip the balance in favor of either humans or bacteria. A clear example of misuse that benefits harmful bacteria is using antibiotics to treat viral infections. Antibiotics do not work on viruses because viruses lack the target structures that these drugs act on. Therefore, conditions like influenza or COVID-19 cannot be treated with antibiotics. Instead, antibiotics can quickly destroy a large portion of our own symbiotic bacteria – organisms that protect us from pathogens and support our overall health. Using antibiotics for viral infections not only fails to eliminate the virus but also weakens our immune defenses.

However, antibiotic misuse does not just stop there. Choosing the wrong antibiotic can have similar negative effects. An ineffective antibiotic may fail to target harmful bacteria while still killing off beneficial microbes. Even when the correct antibiotic is prescribed, using it at the wrong dose can result in only partial elimination of the pathogen. The surviving bacteria may then develop resistance to the drug, rendering it useless for future treatment. A course of antibiotic treatment usually lasts 7–10 days (with some exceptions, such as azithromycin therapy at a dose of 500 mg, which is shorter). Stopping treatment early, before the doctor-recommended duration, can have the same effect as taking an incorrect dose. For these reasons, antibiotics should always be used exactly as prescribed by a medical professional.

Rational antibiotic use is closely tied to the issue of antibiotic resistance and biological wastewater treatment. The biological reactor in a wastewater treatment plant is like a large pool where wastewater from our homes and workplaces flows in. Inside, it mixes with activated sludge to form a gray, foul-smelling suspension containing everything from food scraps and bathwater to living and dead bacteria from our intestines, pathogens, antibiotics, and their metabolites.



Microorganism growth:
A, B) environmental microorganisms growing on a Petri dish with solid broth medium,
C) bacteria capable of *hemolysis* (red blood cell breakdown) growing on a Petri dish with sheep blood added,
D) activated sludge

This environment is essentially a "hotspot" for antibiotic resistance. The horizontal gene transfer processes outlined above occur on a massive scale in this setting. If resistance genes are acquired by harmless bacteria – those responsible for cleaning the wastewater – it might not seem like a problem at first. However, these harmless bacteria can then pass their resistance genes on to pathogenic bacteria, which may then escape into natural water systems along with treated wastewater.

Even more concerningly, there is a strong likelihood that wastewater treatment plants release a variety of antibiotic resistance genes into rivers, lakes, and eventually the Baltic Sea. In aquatic environments, genetic exchange between bacteria is common. It is easy to imagine how these resistance genes could spread to pathogenic bacteria, posing serious risks to the health of both humans and animals.

Better to prevent than to treat

In one of our experiments, we investigated the effects of three antibiotics commonly found in wastewater treatment plants: oxytetracycline, ciprofloxacin, and clarithromycin. The study focused on the efficiency of nitrogen compound removal and the potential transfer of antibiotic resistance genes among activated sludge bacteria under different concentrations of these drugs. We specifically examined an anaerobic process called *anammox* (anaerobic ammonia oxidation), which converts ammonium nitrogen into its gaseous form. At low concentrations (0.001 mg/l), these antibiotics did not inhibit the activity of nitrogen-transforming bacteria. However, and importantly, even at this concentration, the diversity of anammox bacteria in the activated sludge decreased. This is a key finding, as microbial diversity is crucial for maintaining stability in biological systems. Lower diversity makes microbial communities more susceptible to disruption from external factors.

When the antibiotic concentrations were increased (to 100 mg/l), the harmful effects on anammox bacteria became more pronounced, with a clear reduction in their community. Despite the relatively low levels tested, there was a noticeable increase in the number of antibiotic resistance genes for the antibiotics being studied. Further analysis showed that oxytetracycline, in particular, was responsible for reducing the number of anammox bacteria while simultaneously increasing the number of resistance genes for this antibiotic. Interestingly, the findings also suggested that otherwise harmless anammox bacteria might contribute to the spread of resistance to macrolides. Because resistance genes can transfer from non-pathogenic to pathogenic bacteria, it is essential to consistently monitor the presence and concentration of antibiotics and resistance genes in wastewater treatment plants.

Antibiotic resistance and its spread in the environment are significant challenges of our time, and we bear much of the responsibility. Irrational antibiotic use and improper disposal of leftover medications – such as discarding them in the trash instead of returning them to pharmacies for proper disposal – have exacerbated the issue. The consequence of this careless overuse is the emergence of bacteria resistant to an increasing number of antibiotics. Meanwhile, efforts to develop new antibiotics have so far yielded limited results. So, what can we do? As in any battle, strategy is key. Prevention is better than cure. Rational antibiotic use, public education about the spread of resistance, monitoring antibiotic levels and resistance genes in the environment, and developing effective biotechnologies to remove these compounds from wastewater are critical steps toward winning this microbiological war. ■

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